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Target Organ Toxicity: The Blood and Bone Marrow Introduction

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In recent years there has been rapid growth in the sophistication of investigative approaches in hematology primarily associated with the study of leukemogenesis and blood dyscrasias in man and closely allied with the evaluation of antineoplastic drugs. The purpose of this conference was to encourage a similar approach to the study of chemically induced myelotoxicity by promoting discussion among investigators, toxicologists and clinicians sharing a common interest in the blood and bone marrow as targets for toxicity.

The blood and bone marrow represent a widely dispersed organ system comprised of a heterogeneous population of cells. The cellular constituents of the blood and bone marrow are divided into several different lines or pathways of differentiation resulting in the production of mature erythrocytes, granulocytes, lymphocytes, macrophages, and thrombocytes. Chemical agents may demonstrate selective toxicity to individual cell types, cells in particular stages of differentiation, or may be toxic to all hematopoietic cells. Exposure to toxic agents may result in an alteration in the number of cells, their function, or both. Myelotoxic insult can be manifested through destruction of the formed elements of the blood, inhibition of mature cell function, destruction of precursor cells or impaired cell growth and regulation. Impairment may be transitory, in the case of drug-induced leukopenia, or permanent, with the onset of aplastic anemia. As a result of the complexity of these processes and the inherent resiliency of the hematopoietic regulatory process, real or potential risks are often difficult to assess.

A characteristic contributing to the sensitivity of the hematopoietic system to chemical insult is the high rate of cell turnover and DNA synthesis. The importance of understanding the nature and complexity of cell kinetics in conferring susceptibility on hematopoietic cells emerges as a recurring theme in many of the presentations. Drs. Mangalik and Robinson, in their paper, contrast the hematopoietic system, along with the gastrointestinal mucosa and skin, to most other organ systems which are comprised of differentiated cells with limited or no capacity for self-renewal. Dr. Heddle underscores the role of cell division in the expression of chromosomal damage and illustrates the sensitivity and the accuracy of the micronucleus test as a nonspecific indicator of mutagenic damage. In presentations by Drs. Heddle, Lee, Marsh, Dritschilo, and me, it is repeatedly illustrated that there are significant variations in the susceptibility of proliferating cells to chemical or radiation insult as a function of cell cycle phase at the time of exposure.

I describe the application of flow cytofluorometric DNA analysis for use as a sensitive indicator of cycle specific changes in bone marrow proliferative activity occurring as a consequence of exposure to agents such as benzene. Drs. Lee, Kocsis, and Snyder discuss the utility of using ferrokinetics to assess erythropoietic toxicity and to determine the effects of chemical exposure on various stages of differentiation in erythroid cells. They have found it to offer increased sensitivity over routine morphometric methods. Drs. Mangalik and Robinson and Marsh point out that the kinetics of granulopoiesis and release of mature granulocytes are more com-

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plex than described for the erythroid line, primarily due to a variety of factors governing circulating neutrophil numbers such as margination and bone marrow reserve.

Because of these factors, as well as the relatively long lifespan of erythrocytes in the peripheral blood, the general consensus was that peripheral blood counts are insensitive and often inaccurate reflections of hematopoietic activity. Most toxicologists routinely employ peripheral hematology counts as an indicator of myelotoxicity; however, assessments of early effects of chemical agents on the hematopoietic system are better made in the context of bone marrow precursor cell number and turnover.

Our knowledge of stem cell origin and function has rapidly grown in sophistication over the past few years. Drs. Mangalik and Robinson review advances in the development of stem cell assays including the recently described Dexter technique for the culture of hematopoietic cells. Dr. Marsh discusses the potential advantages of the agar diffusion chamber precursor cell assay (ADCPC) for assessing the effects of chemical agents on granulocyte and macrophage precursor cells. The ADCPC assay is an *in vivo* modification of the CFC-C culture technique in which the effects of chemical agents can be assessed on clonigenic cells *in vivo*. These assay procedures have seen only limited use in the past for the evaluation of environmentally induced myelotoxicity, but are now being used with increasing frequency to determine the toxic potential of chemicals toward bone marrow stem cell populations.

Drs. Rickert and Nebert call attention to the importance of pharmacokinetic, disposition, and metabolism studies in evaluating the myelotoxic potential of a chemical agent. In addition to outlining some of the special problems encountered in the analysis of blood and bone marrow samples, Dr. Rickert describes the usefulness and limitations of newer sophisticated instrumentation such as gas chromatography-mass spectroscopy and high performance liquid chromatography. These techniques have the requisite specificity and sensitivity to overcome some of the challenges presented in the analysis of small tissue samples such as bone marrow. Dr. Rickert describes their application to the analysis of benzene and its metabolites in blood and bone marrow. Dr. Nebert emphasizes the importance of dose and route of administration in interpreting genetically controlled differences in susceptibility to chemically induced bone marrow toxicity in different strains of mice. He illustrates this point with a description of differences in the inducibility of mixed function oxidase activity controlled by the Ah locus which gave rise to different patterns of toxicity in response to benzo[a]pyrene administration. This presentation points to the potential importance of genetically determined heterogeneity in governing the response to environmental stimuli, a concept again borne out in Dr. Piomelli's discussion of erythrocyte G6-PD deficiency in man.

Drs. Adamson and Sieber review our current knowledge of the phenomena associated with chemically induced leukemia in man. They emphasized the critical lack of appropriate animal models for the study of human leukemogenesis. Although a number of chemical agents have been recognized to produce leukemia or lymphoma in experimental animals, the characteristics and progression of these malignancies in rodents do not closely resemble those observed in man. These differences seriously cloud the extrapolation of experimental data to humans.

Detailed aspects of studying chemical toxicity to the granulocyte are discussed by Dr. Marsh, who reviews the structure, kinetics, and function of the polymorphonuclear leukocyte and its role in mediating inflammatory response and phagocytosis of microorganisms. Specific examples of chemotherapeutic agents which possess different activities toward granulocyte precursor cells were presented. In the context of presenting recent clinical findings related to combined radiation and chemotherapeutic modalities, Dr. Dritschilo reviews important differences in myelotoxic response between individual and concurrent radiation or chemical exposure. His presentation calls our attention to the potential significance of biologic interactions in evaluating environmentally induced myelotoxicity. Dr. Piomelli presents examples of two different types of chemical toxicity to the red cell. A direct effect on mature erythrocytes brought about by a genetically determined deficiency in G-6-PD leading to an increased susceptibility to drug-induced hemolysis and the effects of lead on hemoglobin synthesis and iron incorporation into protoporphyrin.

Dr. Loose outlines the role of the macrophage in phagocytosis as well as initiation and amplification of the immune response. He reminds us of the underlying relevance of alterations in leukocyte and macrophage function, namely, that suppression of specific cell function does result in an increased susceptibility to bacterial, viral, or parasitic infection as well as increased susceptibility to acceptance of tumor transplants in syngeneic hosts. Dr. Loose also illustrates the complexity involved in evaluating alterations in cell function by distinguishing between a direct biochemical effect on

macrophage phagocytic ability and an indirect effect on macrophage phagocytic response via interference with the serum opsonin, fibronectin. Although the lymphocyte is more often considered within the context of immunology, its origin and role within the hematopoietic system render it an important aspect of any discussion concerning hematologic response to toxic agents. Drs. Silkworth and Loose, in their contributed paper, provide an overview of approaches available for the study of lymphocyte function and discuss factors to be considered in the assessment of chemically-induced immunotoxicity.

In the past, toxicologic investigations of potentially myelotoxic agents have been largely restricted to analysis of hemograms and the numbers of circulating peripheral blood elements. Recent advances in experimental hematology have resulted in an increased awareness of the importance of understanding the dynamics of hematologic regulation and function and have provided the toxicologist with new and sensitive tools for the study of induced myelotoxicity.